
Cleaning of rotary nickel–titanium endodontic instruments

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Abstract

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Aim To develop and evaluate an effective cleaning procedure for rotary nickel–titanium (NiTi) endodontic instruments.

Methodology New rotary instruments (ProFile size 25/.04) were contaminated by preparing canals of extracted teeth. Three factors were evaluated to develop an effective cleaning sequence: dry or moist storage before cleaning; mechanical removal (brushing); and chemical dissolution in 1% NaOCl with ultrasonication. Debris on flutes was scored after staining *in situ* with Van Gieson's solution at $\times 45$ magnification. Debris was classified as stained or unstained particulate debris and organic film, and rated as none, slight, moderate or heavy. The effectiveness of a recommended cleaning sequence was tested on different instrument types and in private endodontic practices.

Results All new instruments showed metallic spurs and fine particulate debris on the surfaces. After contamination, brushing alone removed most particulate debris, but did not remove organic film. NaOCl effectively removed organic film. Under laboratory conditions, the sequential cleaning procedures (moist storage, brushing followed by immersion in 1% NaOCl and ultrasonic cleaning) totally removed organic debris. Dry storage before cleaning or autoclaving with debris present reduced cleaning effectiveness ($P < 0.001$, one-way ANOVA). In three private practices, the cleaning protocol substantially reduced biological contamination, but complete cleaning was not always achieved (87% clean).

Conclusion Complete removal of organic debris from instruments is feasible using a combination of mechanical removal and chemical dissolution, but requires meticulous attention to details.

Keywords: cleaning, cleaning of NiTi instruments, cross-infection, endodontic instruments, nickel–titanium.

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Introduction

Cross-infection control is a major issue in the dental care setting because of concerns about transmission of disease via the oral cavity. Endodontic treatment may directly involve contact with saliva, blood and infected pulp tissue. The US Centers for Disease Control and Prevention (Centers for Disease Control 1987) emphasized that all blood and body fluids that have been implicated in transmitting blood-borne infections should be considered as potentially infectious, regardless of a patient's

infectious status. According to Australian National Health and Medical Research Council (NHMRC) guidelines (2002), instruments used in invasive dental procedures (including root canal treatment) are considered to involve a critical site, and should be sterile at the time of use.

Several studies have shown that rotary nickel–titanium (NiTi) instruments can be of multiple use without intracanal failure (Yared *et al.* 2000, Gambarini 2001, Svec & Powers 2002). Manufacturers recommend discarding instruments after a specified number of uses or whenever visible deformation is observed. Several factors must be considered such as instrument design, size and stress produced during instrumentation of complex and curved canals (Sattapan *et al.* 2000).

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Effective reprocessing of reusable instruments involves cleaning to remove organic residue and sterilization. Several factors, including the physical properties of the sterilizing agents and debris on the surface of the instruments, can influence the effectiveness of the sterilization process. Organic residue may prevent a disinfectant or sterilant from contacting the instrument being processed and may also bind and inactivate chemical disinfectants (Muscarella 1998). Instruments must be pre-cleaned to remove organic debris before sterilization in order to prevent continuing viability of pathogens (Miller & Sheldrake 1991, Parker & Johnson 1995).

Only a few studies have investigated the effectiveness of cleaning methods for endodontic instruments. Segall *et al.* (1977) recommended chair-side cleaning by wiping endodontic instruments with gauze during use; however, a large amount of debris still remained on the instruments after cleaning. Murgel *et al.* (1990) investigated the effectiveness of various mechanical cleaning methods such as gauze soaked with alcohol, a sponge soaked with alcohol and an ultrasonic bath. They reported that none of these methods was able to clean the instruments totally. Similarly, Eggert *et al.* (1999) and Marending *et al.* (1998) reported that ultrasonic cleaning was an ineffective method to totally remove debris from rotary NiTi instruments.

Sodium hypochlorite (NaOCl) is recognized as an effective disinfectant because of its broad-spectrum antimicrobial activity. In addition, the chemical removal of organic tissue by NaOCl has been reported in many studies (Hand *et al.* 1978, Koskinen *et al.* 1980). The efficacy of NaOCl as a tissue-dissolving and disinfecting agent depends on its concentration and time of exposure (Mentz 1982, Mooror & Wesselink 1982). Generally, concentrations between 0.5 and 5.5% are used clinically as root canal irrigants.

For cleaning of instruments, the strength of NaOCl solutions must be balanced against potential damage to instruments by corrosion. Several investigators have demonstrated the corrosion resistance of NiTi endodontic instruments in NaOCl. Haikel *et al.* (1998) reported that no corrosion defects were observed under scanning electron microscope (SEM) examination after 12- or 48-h immersion in 2.5% NaOCl. Only negligible amounts of titanium were released from the NiTi instruments after immersion in ultrasonicated 1% NaOCl solution for 1 h (Busslinger *et al.* 1998).

To date, no cleaning method has been demonstrated to clean NiTi endodontic instruments totally. The purpose of this study was to evaluate the effectiveness of various cleaning procedures, using combined mechanical and

chemical techniques, in removing debris. A further aim was to develop a simple cleaning sequence that could be readily incorporated into clinical practice. The desired end point of the study was an instrument devoid of organic material (within limits of detection), so that it could be considered free of biological risk.

Materials and methods

Overview

Rotary NiTi instruments were contaminated by preparing canals of extracted teeth. The effectiveness of a number of cleaning procedures was evaluated separately and in combination until a sequence of steps was developed to result in removal of all detectable organic debris. The cleaning sequence was then evaluated under conditions of private practice. Debris was scored over the entire surfaces of the flutes after staining with a histological stain.

Instruments

New rotary NiTi instruments (ProFile, size 25 with .04 taper, 25 mm; Dentsply Maillefer, Ballaigues, Switzerland) were used for most experimental work. Other sizes (15–40) and instrument types (Flexmaster; VDW GmbH, München, Germany, K3; NT Company, Chattanooga, TN, USA, Quantec; NT Company, Chattanooga, TN, USA) were also evaluated after the cleaning protocol was developed. Instruments were grasped by the handle with tweezers to avoid contamination. Instruments were stored in covered Petri dishes except during the cleaning and scoring procedures to minimize exposure to dust or exogenous debris. Before the cleaning procedures, instruments were used under simulated clinical conditions to prepare canals of extracted teeth in order to produce a build-up of organic material on the instruments.

Scoring system

The entire surface of the flutes of each instrument was examined at $\times 45$ magnification using a dissecting microscope (American Optical Corporation, Buffalo, NY, USA). All instruments were first immersed in Van Gieson's solution for 3 min in order to stain any biological debris. The instruments were then rinsed using distilled water and air-dried on an endodontic stand (ProFile instrument stand; Dentsply, Maillefer, Ballaigues, Switzerland). Van Gieson's solution was used because it stains collagen effectively (Carson 1990),

and a substantial proportion of the debris was likely to consist of dentine filings which contain collagen. The stain also highlighted other organic material, including a thin film often noted on the surface of the flutes.

To resist movement of the instruments during examination, a specially designed holder was used. The holder consisted of a rectangular hollow block, square in cross-section with an insert of rubber impression material, to accept the instrument handle in order to keep the instrument horizontal during examination. The experimental instruments were coded and examined at $\times 45$ magnification. Each instrument was examined for debris at three levels: apical, middle and coronal. At each level, the instruments were examined on four sides by sequentially rotating the block through 90° .

Material on the instruments was classified as stained or unstained particulate debris, or as a thin film stained by the Van Gieson's solution. The category and extent of debris were recorded using the criteria shown in Table 1 and illustrated in Fig. 1.

Only one category of debris was assigned to each site examined, with stained debris ranking ahead of the presence of organic film and film ahead of unstained debris. Hence, if an instrument contained stained debris, film and unstained debris, it was rated as 'stained debris'. This categorization was based on an estimate of 'biological risk'. Stained material was considered to be a biological risk factor, so that it should be ranked ahead of unstained material, which is presumably not of biological origin. Stained debris was ranked ahead of organic film because of its greater bulk.

A semiquantitative estimate of the 'biological burden' present on each instrument was then made (see data analysis below), and each instrument was also classified as positive or negative for 'biological risk' (defined as the presence of stained material anywhere on the instrument).

Evaluation of different cleaning procedures

Baseline levels of contamination

Forty new instruments were stained immediately after removal from their packages. Twenty instruments were contaminated by using them to prepare canals of extracted teeth, and then stained after dry storage overnight, without any cleaning procedure. Another 20 instruments were contaminated by the same method, then inserted into a sponge saturated with 0.1% chlorhexidine gluconate aqueous solution for 2 h to keep them moist (and to achieve initial cleaning), and stained in the same manner as the dry storage group.

Experimental groups

Eighty new instruments were used to instrument canals of extracted teeth. After visual debris was noted, all instruments were inserted into a sponge soaked in 0.1% chlorhexidine gluconate aqueous solution for 30 min. The instruments were then randomly assigned into four equal groups representing four different cleaning procedures.

- Group I: The instruments were placed in an instrument stand (ProFile instrument stand; Dentsply Maillefer, Ballaigues, Switzerland), and the flutes were brushed for 20 strokes per row with a nylon bristle brush (a small bottle brush or test tube brush) under running distilled water. The stand allowed brushing without risk of sharp injury.
- Group II: Instruments were brushed as in group I, and then placed in a beaker containing 1% NaOCl (diluted household bleach; Homebrand, Grocery Wholesalers Pty Ltd, Yennora, NSW, Australia), soaked for 10 min and rinsed under running distilled water.
- Group III: Without brushing, the instruments were directly immersed in a beaker containing 1% NaOCl for

Table 1 Scoring system for debris on instruments

A Categories of debris

SD (stained particulate debris): Particulate matter stained red or orange

F (organic film): A thin unstructured layer covering part of the instrument surface and generally stained red

UD (unstained particulate debris): Fine particles that did not exhibit any red/orange colouration after staining

C: Clean

B Extent of stained debris

0 (none)

1 (film only)

2 (slight): Scattered particles spaced widely apart on the flute surfaces

3 (moderate): Numerous particles with areas of continuous coverage of surfaces

4 (heavy): Areas of the instruments where the flutes were packed with debris to their entire depth

Film was scored as absent or present (1) only, regardless of extent.

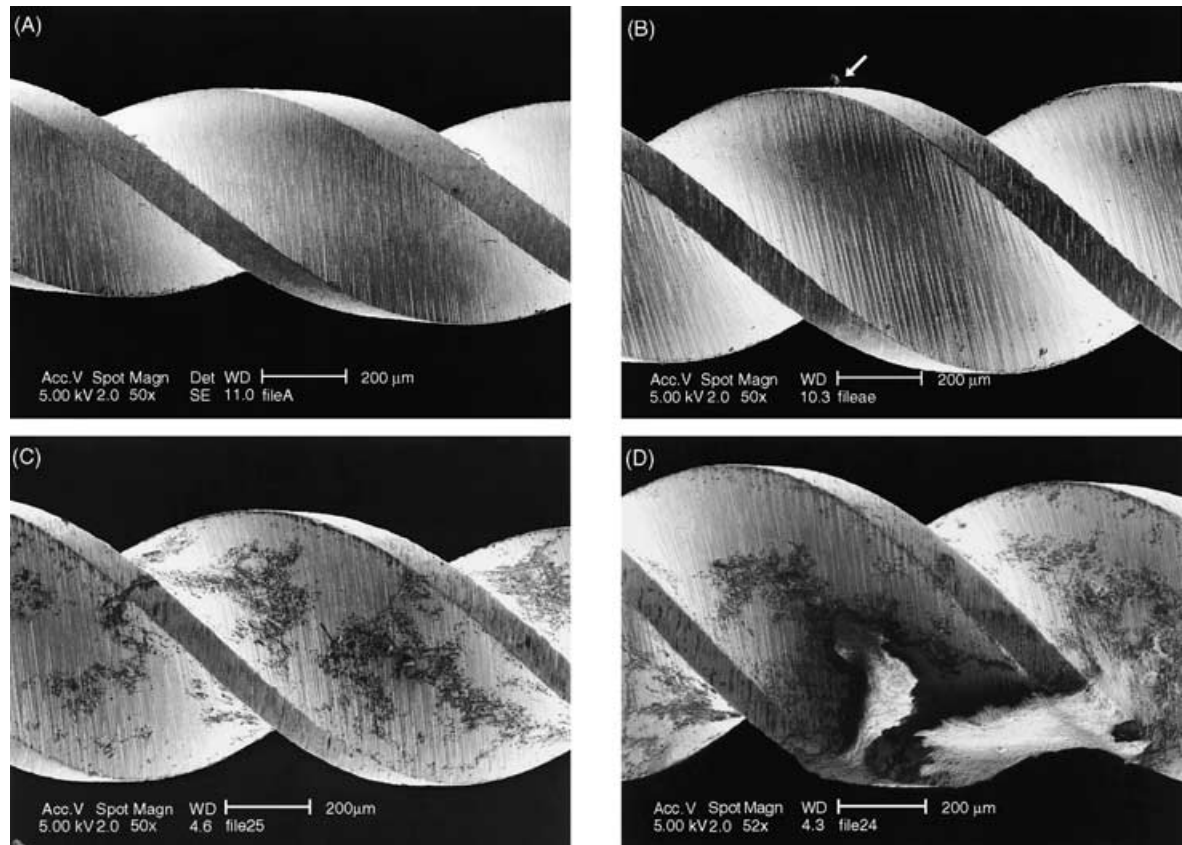


Figure 1 Categories of debris present on instruments (SEM photographs, $\times 50$ magnification). The extent of manufacturing features (milling grooves and swarf) is evident in all instruments. (A) Clean instrument. (B) Unstained debris, metal particle (arrow). (C) Organic film. During processing for SEM, the thin film tends to aggregate into an irregular layer on the instrument surface. (D) Stained particulate debris. The instrument shows areas of contamination with stained debris plus areas of organic film.

10 min, and then the beaker was placed into an ultrasonic bath (Health Sonics Corporation, Livermore, California, USA) for 5 min and rinsed.

- Group IV: Instruments were brushed, immersed for 10 min in 1% NaOCl, placed in the ultrasonic bath for 5 min and rinsed.

After the cleaning procedures, instruments were air-dried and immersed in Van Gieson's solution for 3 min, rinsed under running distilled water and air-dried on the endodontic stand. Instruments were then scored for debris at $\times 45$ magnification as described above.

Based on the above experiments, a complete cleaning sequence that effectively removed all stained debris was adopted. The sequence consisted of (i) brushing with a nylon brush for 20 strokes; (ii) immersion in 1% NaOCl for 10 min; and (iii) ultrasonication in the same solution for 5 min, with final rinsing.

Effects of storage conditions or autoclave sterilization on cleaning effectiveness

One hundred instruments from the previous experiments were cleaned using the complete cycle, confirmed to be free of stained material, and were reused to instrument canals of extracted teeth. After visual debris was obtained, the instruments were randomly divided into five equal groups:

- Group 1 (dry-storage baseline): The instruments were bench-dried overnight.
- Group 2 (moist storage): The instruments were kept moist by placing them overnight in a sponge soaked in 0.1% aqueous chlorhexidine gluconate, with no additional cleaning.
- Group 3 (dry storage, complete cleaning sequence): The instruments were bench-dried overnight and

were then transferred to the brushing stand, and subjected to the complete cleaning sequence outlined above.

- Group 4 (moist storage, complete cleaning sequence): The instruments were inserted into a sponge soaked in 0.1% chlorhexidine gluconate aqueous solution. The instruments were then transferred to the brushing stand and cleaned as above.

- Group 5 (autoclave sterilization group): Instruments were brushed on the instrument stand as described earlier and passed through autoclave sterilization (Gettinge, Bulimba, Brisbane, Australia) at 134 °C, 220 kPa for 3 min. The instruments were then cleaned with the full cleaning protocol.

All instruments were stained with Van Gieson's solution and scored in the same manner as before.

Evaluation of cleaning protocols in endodontic practices

Three private endodontic practices participated in this study. Ten ready-to-use instruments were randomly collected from each dental practice, after the instruments had been subjected to the cleaning and sterilization procedure currently used in the practice. Instruments were stained with Van Gieson's solution, and scored using the same technique and scoring system as in the previous experiments. The data were collected as baseline information.

Clearly defined cleaning instructions as well as all required cleaning equipment and chemical solutions were supplied to the participating practices. Each endodontist was requested to use 20 new rotary NiTi instruments of assorted sizes and types as they would usually use for canal preparation in patients. After canal instrumentation, all instruments were placed in a moist sponge and cleaned by the dental assistants using the protocol provided. They were asked to strictly follow instructions, but rinsing was done with tap water rather than distilled water. At no time was the instrument blade to be touched. After cleaning, instruments were transferred to an endodontic stand, and collected for staining and scoring.

Scanning electron microscope examination

A number of instruments from each experiment were examined under SEM at various magnifications. Photographs were taken to show the structure and extent of debris accumulation on the surface of the instruments.

Data analysis

Two different rating systems were used. In the first, the extent of material remaining on the instrument was scored, to provide an estimate of the effectiveness of debris removal associated with each component of the cleaning protocol. In the second, each instrument was considered as a unit on the basis that remaining material of biological origin anywhere on the instrument poses a potential risk for cross-infection.

Extent of biological burden

This calculation was based on an assessment of the quantity of stained organic material present on the instrument. Because Van Gieson's solution can stain biological material effectively, unstained debris was considered not to pose a biological risk and was regarded as equivalent to a clean surface.

Each instrument was assessed at 12 sites, which encompassed the entire cutting area of each instrument. A rank score was given to each site depending on the extent of biological contamination (Table 1). The scores from all sites on each instrument were summed. The minimum score for each instrument was 0 (no stained material present), and the maximum score was 48 (all surfaces heavily contaminated with particulate debris). The mean score for each instrument was calculated and then converted into the mean percentage of maximum biological contamination (MBC).

Biological risk analysis

Considering each instrument as one unit, the instrument was classified as either positive (stained debris or film present) or negative (unstained debris only or clean) 'biological risk'. The presence of stained material in any location of the instrument was considered to constitute a 'biological risk'.

Statistical analysis

Univariate ANOVAs were conducted to examine the effectiveness of the cleaning procedures. All paired comparisons between means were performed at the 0.05% level of significance, using the least significant difference (LSD). In cases where the variances were clearly unequal, a logarithmic transformation was applied to the data before the ANOVA was performed. Means equal to zero were excluded from the ANOVA.

Table 2 Baseline extent of biological contamination

| | <i>n</i> | Number clean ^a | Mean percentage of MBC | SE ^b |
|---------------------|----------|---------------------------|------------------------|-----------------|
| New instruments (N) | 40 | 38 | 0.6 | 0.1 |
| Dry storage (D) | 20 | 0 | 52 | 0.9 |
| Moist sponge (M) | 20 | 0 | 31 | 0.4 |

The extent of biological burden (expressed as a percentage of maximum contamination) is given for new instruments before and after canal preparation.

F-test: $P < 0.001$.

Paired comparisons: N versus D, $P < 0.05$; N versus M, $P < 0.05$; D versus M, $P > 0.05$.

^aNo stained debris or film detected, but unstained debris may be present.

^bSE, standard error of the mean.

Results

Baseline

All 40 new instruments, direct from their package, contained unstained debris, and two instruments were slightly contaminated with organic material, which was stained with Van Gieson's solution. Under SEM examination, metal particles and unidentified debris were found. Instrumentation of root canals of extracted teeth resulted in substantial accumulation of debris on the instruments, which was often limited to a particular area of the flutes. The MBC for each instrument was much higher than new instruments from their packages (52% compared to 0.6%; Table 2), and all instruments contained organic material. Inserting instruments into a sponge soaked with 0.1% chlorhexidine gluconate aqueous solution resulted in a significantly lower debris score, indicating a mechanical cleaning effect in addition to keeping debris moist (MBC = 31%; $P < 0.001$, one-way ANOVA; Table 2).

Evaluation of different cleaning procedures

The data obtained from different cleaning methods after moist storage are summarized in Table 3 and Fig. 2. All steps in cleaning were partially effective in removing debris, and only light accumulation was recorded after all cleaning procedures were applied individually.

Mechanical removal by brushing alone reduced the level of debris remaining on the instruments. MBC was lower in the brushing-only group (15%) compared to the wet storage group (31%; $P < 0.001$, one-way ANOVA). Brushing alone did not eliminate organic film, which was observed on 8 of 20 instruments. Organic film was successfully removed only when 1% NaOCl was used as an adjunct to mechanical debris removal by brushing. With the sequence of combined mechanical and chemical removal (brushing, NaOCl and ultrasonic bath), all organic (stained) material was totally removed (MBC = 0%). Totally clean instruments were observed in 17 of 20 instruments, and only small amounts of unstained debris were observed in 3 of 20 instruments. Without mechanical removal by brushing prior to NaOCl immersion and ultrasonic bath, small amounts of stained debris and organic film remained on the instruments, with MBC of 3%. Statistically significant differences were observed between the full cleaning protocol and no brushing prior to NaOCl treatment and ultrasonication group ($P < 0.05$, Student's unpaired *t*-test).

Effects of storage conditions or autoclave sterilization on cleaning effectiveness

The effects of storage conditions on the cleaning procedures are shown in Table 4. The moist storage group with full cleaning protocol (brushing, NaOCl treatment and ultrasonic cleansing) showed similar results to the previous experiment. All organic (stained) material was removed (MBC = 0%, unstained debris on 4 of 20

Table 3 Effect of cleaning procedures on the extent of biological contamination of instruments

| | <i>n</i> | Number clean ^a | Mean percentage of MBC | SE ^b |
|---|----------|---------------------------|------------------------|-----------------|
| Baseline (moist sponge) | 20 | 0 | 31.0 | 0.4 |
| Brushing only (B) | 20 | 0 | 15.0 | 0.4 |
| Brushing plus NaOCl soak (BN) | 20 | 15 | 4.2 | 0.4 |
| NaOCl soak plus ultrasonication (NU) | 20 | 16 | 3.0 | 0.3 |
| Brushing, NaOCl and ultrasonication (BNU) | 20 | 20 | 0.0 | 0.0 |

F-test: $P < 0.001$.

Paired comparisons: B versus BN, $P < 0.05$; B versus BNU, $P < 0.05$; BN versus NU, $P > 0.05$.

^aNo stained debris or film detected, but unstained debris may be present.

^bSE, standard error of the mean.

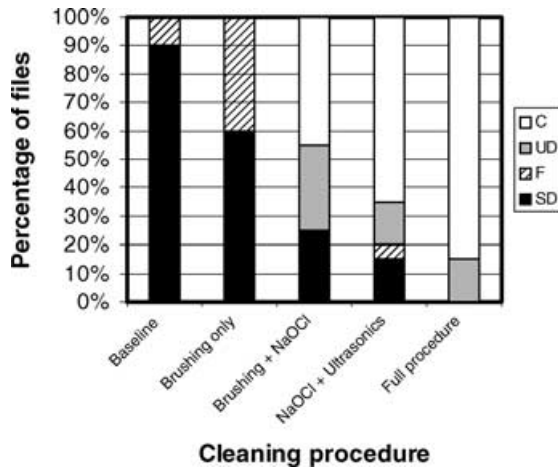


Figure 2 Effectiveness of different cleaning steps on the cleanliness of instruments. SD: Stained particulate debris; F: organic film; UD: unstained particulate debris; C: clean.

instruments). In contrast, the dry storage group demonstrated a small amount of stained debris on some instruments (8 of 20) even after the complete cleaning sequence, with $\text{MBC} = 6.2\%$. Similarly, autoclave sterilization prior to the full cleaning sequence reduced cleaning effectiveness, with stained debris remaining on all instruments and $\text{MBC} = 17\%$. Statistical analysis demonstrated a highly significant difference amongst groups ($P < 0.001$, one-way ANOVA).

Evaluation of cleaning protocol with different instrument types and in endodontic practices

All ProFile and Flexmaster instruments were routinely free of stained debris, but occasional slight debris was found on K3 and Quantec instruments. Ready-to-use, cleaned and sterilized instruments from all three endodontic practices showed measurable contamination.

Table 4 Effect of storage on the effectiveness of cleaning

| | <i>n</i> | Number clean ^a | Mean percentage of MBC | SE ^b |
|------------------|----------|---------------------------|------------------------|-----------------|
| Autoclaving (A) | 20 | 0 | 17.0 | 0.5 |
| Dry storage (D) | 20 | 11 | 6.2 | 0.4 |
| Moist sponge (M) | 20 | 20 | 0.0 | 0.0 |

Each instrument was subjected to the complete cleaning procedure after moist or dry storage or autoclaving (without prior cleaning).

F-test: $P < 0.001$.

Paired comparison: A versus D, $P < 0.05$.

^aNo stained debris or film detected, but unstained debris may be present.

^bSE, standard error of the mean.

Using the complete cleaning protocol devised above, all three practices showed a substantial reduction in biological burden with the new protocol ($\text{MBC} = 2.4\%$ vs. 27.5% ; $P < 0.05$, Student's unpaired *t*-test). None were able to remove organic material completely from all instruments, but the number of clean instruments increased from 10% (3 of 30), with the routine cleaning protocols, to 87% (52 of 60), with the new cleaning protocol.

Discussion

Numerous studies have recommended cleaning instruments between uses, before sterilization, to minimize the risk of cross-infection, even though Johnson *et al.* (1997) showed that bioburden present on endodontic instruments did not affect the autoclave sterilization process. Muscarella (1998) also concluded that heat sterilization is completely effective in killing bacteria and viruses on dental instruments even in the presence of organic debris. Unlike heat, low-temperature sterilization requires direct contact between the sterilant and instrument for effective sterilization. Ideally, all organic materials should be removed by cleaning before sterilization to minimize risk. In the inadvertent absence of effective sterilization, the presence of any organic material on an instrument constitutes a potential risk. This study attempted to detect the presence of biological material anywhere on the flutes of instruments, as well as estimating the total bioburden present on the instrument.

Identification and quantitation of debris on endodontic instruments raises important questions with regard to the potential for cross-infection. SEM is limited in its ability to determine the nature of contaminants, and is impractical as a scoring technique if the entire surface of the instrument is to be examined. Extraction methods followed by chemical analysis (Sanchez & MacDonald 1995) assume that the contaminant is easily and totally removed by the extraction technique. Staining *in situ* with a general histological stain appears to be a practical approach. It has been used previously for evaluating blood contamination of matrix bands, using a forensic stain for haemoglobin (Lowe *et al.* 2002). Van Gieson's stain was used in this study because it is an easy single-step technique to perform and is able to stain strongly a wide range of organic materials, which are considered to be potential biological risk factors. In the present study, it was able to distinguish between unstained and stained particulate material and to highlight a thin organic film on the flute surfaces.

Evaluation of 40 new ProFile instruments directly from their packages revealed metal residue and unstained particulate debris on the surface of every instrument. Two of 40 new instruments (5%) had slight contamination with stained debris. A similar finding has been frequently reported for new NiTi instruments (Marending *et al.* 1998, Eggert *et al.* 1999, Tanomaru Filho *et al.* 2001, Martins *et al.* 2002). Given the occasional presence of biological contamination, new rotary endodontic instruments from their packages should be cleaned before sterilization and clinical use. Zmener & Spielberg (1995) and Tanomaru Filho *et al.* (2001) recommended the ultrasonic bath as the most effective method to remove foreign particles from the surface of the instruments. However, neither study could demonstrate any totally clean instrument after ultrasonic cleaning, with a few sparsely distributed particles remaining on the flutes.

The identity of unstained debris and its biological significance are uncertain. Several sources are possible, during manufacture and packaging, as well as the cleaning procedure itself. Martins *et al.* (2002) reported adherent deposits containing carbon and sulphur, which appeared to originate from lubricating oils used during manufacture. These deposits served as sites for subsequent accumulation of debris during clinical use. The predominance of unstained debris on new instruments and an increase after brushing or washing with tap water suggest that the debris includes dust or fine silica particles, which are almost universally distributed. Washing with distilled water after the complete cleaning sequence reduced the number of instruments that presented with unstained debris to 15% compared to new instruments from their packages (>90%). Unless instruments are cleaned under dust-free conditions using filtered distilled water, it may be impossible to achieve complete removal. Because the material does not appear to have a biological source (or at least is not stained), there is no reason to consider it potentially infectious.

Mechanical cleaning alone removed a significant amount of debris, but could not totally clean the instruments. The MBC was reduced from 31% (moist storage control) to 15% (brushing-only group). Brushing instruments on an endodontic stand was considered to be a safe and effective method, and should reduce the risk of sharp injuries compared to using a bur brush. Only slight debris accumulation and organic film were observed on the instruments after brushing.

Sodium hypochlorite has the ability to dissolve organic material (Hand *et al.* 1978, Koskinen *et al.* 1980), and its tissue-dissolving ability does not depend only

on its concentration. Moorer & Wesselink (1982) demonstrated that mechanical agitation (fluid flow) was an important factor. The results from the present study confirm that the use of an ultrasonic bath combined with 1% NaOCl after brushing was required for removal of all organic materials. Bloomfield & Miles (1979) indicated that the active principle of NaOCl solution was the amount of undissociated hypochlorite (HOCl) molecules. The tissue-dissolving power of NaOCl appears to be strongly dependent on the ratio of hypochlorite to organic matter (Moorer & Wesselink 1982). Our study showed that the combined use of 1% NaOCl and an ultrasonic bath for 5 min could not completely remove organic material from the instruments without prior removal of large quantities of debris by brushing. These results suggest that the sequence of steps in the cleaning protocol is a key factor, and emphasizes the necessity of mechanical removal of debris prior to chemical removal of organic materials by NaOCl.

When instruments were stored dry (overnight) or autoclaved with organic material present, the sequential combined mechanical and chemical cleaning procedures failed to remove organic material completely. Moist storage after clinical use is essential for effective cleaning (Sanchez & Macdonald 1995, Miller 2002), and use of a sponge soaked in chlorhexidine also provided a degree of initial cleaning.

Some instruments could not be totally cleaned with this cleaning protocol, either under laboratory conditions or in private practices. This may be because of their flute designs with wide radial lands and deep narrow flutes. These designs tended to retain debris and cause more difficulty while brushing because the relatively large brush bristles cannot enter the narrower flutes. Localized defects were occasionally observed in some instruments, which may be manufacturing defects or may possibly be created during canal preparation. Debris retention was commonly observed in these defects.

Cleaned, ready-to-use instruments (subjected to the normal cleaning procedure) from three endodontic practices showed varying degrees of debris accumulation on the surface of the instruments. This highlights the ineffectiveness of existing cleaning procedures even in specialist practices. Wiping with gauze and alcohol (Segall *et al.* 1977), bur brushing and hand scrubbing were operator-sensitive. Even with the addition of ultrasonic cleaning, debris still could not be totally removed from the instruments. This confirms the results from the laboratory study, which showed that mechanical cleaning alone could not completely remove debris from the instruments.

With the cleaning sequence prescribed following our experimental study, its use in three practices revealed slight residual contamination of a small percentage of instruments. This result can be interpreted in several ways. The cleaning technique may be too time-consuming and operator-sensitive, which may not be practical in busy endodontic practices. Another explanation is that the cleaning procedures might not be performed correctly because only instructions were provided without demonstrating the procedure at each practice. Moreover, each practice used different types of instruments, some of which were difficult to clean. However, the results from the three private endodontic practices confirmed the ability of the cleaning protocol to reduce the extent of biological contamination on the instruments and render most instruments clean. The MBC was reduced from 27.5% (ready-to-use instruments) to 2.4% (instruments cleaned with the cleaning protocol), while the number of clean instruments increased from 10 to 87%.

Generally, no effect of NaOCl was observed on the surface of the experimental instruments, confirming the finding of several studies that reported the corrosion resistance of NiTi (Busslinger *et al.* 1998, Haikel *et al.* 1998, Stokes *et al.* 1999). Only localized defects on the surface of the instruments showed possible evidence of corrosion, but the presence of stained debris within defects suggested that they were present at the time of initial use within the canal. Stokes *et al.* (1999) speculated that manufacturing factors affect the corrosion of endodontic instruments.

Conclusions

All new instruments from their packages should be cleaned and sterilized before clinical use. After use, instruments should be kept in moist storage while awaiting the cleaning process. Sequential combined mechanical and chemical cleaning procedures could not totally remove organic materials from all types of instruments. In private endodontic practices, a small percentage of instruments showed slight residual contamination at a microscopic level. However, under carefully controlled conditions, most instruments were totally cleaned of organic debris with this sequential combined mechanical and chemical cleaning procedure.

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